

IN THE CLAIMS

1. (previously presented) A method of making a plant artificial chromosome, comprising:

(a) preparing recombinant protoplasts of a first plant species containing an exogenous nucleic acid;

(b) producing chromosome fragments of chromosomes contained in the recombinant protoplasts;

(c) fusing the recombinant protoplasts of (b) with protoplasts of a second plant species to produce fused protoplasts, wherein the first and second plant species may be the same or different; and

(d) identifying fused protoplasts of (c) or cells derived from the fused protoplasts of (c) that contain chromosome fragments containing the exogenous nucleic acid, and that exhibit normal plant chromosomal activities.

2. (original) The method of claim 1 wherein (b) comprises irradiating the protoplasts.

3. (original) The method of claim 1 wherein (b) comprises contacting the protoplasts with a chemical agent.

4. (original) The method of claim 3 wherein the chemical agent is calicheamicin, esperamicin, dynemicin or neocarzinostatin.

5. (original) The method of claim 1 wherein said identifying of (d) comprises pulsed field gel electrophoresis.

6. (original) The method of claim 1 wherein said second plant species is the same as said first plant species.

7. (original) The method of claim 1 wherein said second plant species is a member of the same family as said first plant species.

8. (original) The method of claim 1 wherein said first plant species is a monocot.

9. (original) The method of claim 1 wherein said first plant species is a dicot.

10. (original) The method of claim 1 further comprising (e) regenerating a whole plant from the recombinant protoplasts of (a), prior to (b).

11. (original) The method of claim 1 further comprising (f) regenerating a whole plant from the fused protoplasts or plant cells identified in claim 1(d).

12. (original) The method of claim 1 wherein the exogenous nucleic acid comprises at least one recombination site.

13. (original) The method of claim 1 wherein the exogenous nucleic acid comprises at least one restriction site.

14. (original) The method of claim 1 wherein the exogenous nucleic acid comprises at least one coding region.

15. (previously presented) The method of claim 1 wherein the exogenous nucleic acid comprises at least one sequence comprising a yeast chromosomal element.

16. (currently amended) The method of claim 1 wherein the exogenous nucleic acid comprises a yeast artificial chromosome.

17. (cancelled)

18. (previously presented) A method of preparing a transgenic plant comprising:

(a) preparing recombinant protoplasts of a first plant species containing an exogenous nucleic acid;

(b) producing chromosome fragments of chromosomes contained in the recombinant protoplasts;

(c) fusing the recombinant protoplasts of (b) with protoplasts of a second plant species to produce fused protoplasts, wherein the first and second plant species may be the same or different;

(d) identifying fused protoplasts of (c) or cells derived from the fused protoplasts of (c) that contain chromosome fragments that exhibit normal plant chromosomal activities; and

(e) regenerating a whole plant from the protoplasts or cells identified in (d) that contain said chromosome fragments containing the exogenous nucleic acid, and that exhibit normal plant chromosomal activities.

19. (previously presented) A method of making a plant artificial chromosome, comprising:

(a) producing transformed plants of a first plant species containing an exogenous nucleic acid;

(b) producing chromosome fragments of chromosomes of said first plant species;

(c) crossing said first plant species containing the chromosome fragments with a second plant species to produce hybrid plant species wherein said first and second plant species may be the same or different; and

(d) identifying hybrid plant species of (c) or cells or protoplasts thereof containing at least one chromosome fragment containing the exogenous nucleic acid, and that exhibits normal plant chromosomal activities.

20. (currently amended) Isolated plant cells or protoplasts containing at least one plant chromosome fragment exhibiting normal chromosomal activities produced by the method of claim 1, wherein the chromosome fragment contains the exogenous nucleic acid.

21. (original) A culture of protoplasts or plant cells identified in claim 1(d).

22. (original) A whole plant produced by the method of claim 11.

23. (original) The whole plant of claim 22 that is a monocot plant.

24. (original) The whole plant of claim 22 that is a dicot plant.

25. (original) Seed derived from the whole plant of claim 22.

26. (original) The whole plant regenerated by the method of claim 18.

27. (original) A plant cell culture derived from the whole plant of claim 25.

28. (original) Seed derived from the whole plant of claim 26.

29. (currently amended) A hybrid plant species or cells or protoplasts thereof containing at least one chromosome fragment that exhibits normal plant chromosomal activities, produced by the method of claim 19, wherein the chromosome fragment contains the exogenous nucleic acid.

30. (previously presented) A recombinant nucleic acid comprising a first centromeric sequence functional in a plant cell, and a second centromeric sequence functional in a yeast cell, wherein said recombinant nucleic acid exhibits normal plant chromosomal activities.

31. (original) A recombinant vector comprising the nucleic acid of claim 30.

32. (original) The recombinant vector of claim 30 that is a shuttle vector.

33. (original) A recombinant cell comprising the nucleic acid of claim 30.

34. (original) The recombinant cell of claim 33 which is a plant cell.

35. (original) The recombinant cell of claim 33 which is a yeast cell.

36. (previously presented) The method of claim 15, wherein the yeast chromosomal element comprises a first centromeric sequence functional in a yeast cell.

37. (previously presented) The method of claim 36, wherein the chromosome fragments further comprise a second centromeric sequence functional in a plant cell.

38. (previously presented) A method of making a plant artificial chromosome, comprising:

(a) preparing recombinant protoplasts of a first plant species containing an exogenous nucleic acid comprising a selectable marker gene;

(b) producing chromosome fragments of chromosomes contained in the recombinant protoplasts;

(c) fusing the recombinant protoplasts of (b) with protoplasts of a second plant species to produce fused protoplasts, wherein the first and second plant species may be the same or different; and

(d) identifying fused protoplasts of (c) or plant cells derived therefrom that contain chromosome fragments that contain the exogenous nucleic acid and that exhibit normal plant chromosomal activities.

39. (previously presented) The method of claim 38, wherein the exogenous nucleic acid further comprises at least one yeast chromosomal element.

40. (previously presented) The method of claim 39, wherein the yeast chromosomal element comprises a first centromeric sequence functional in a yeast cell.

41. (previously presented) The method of claim 40, wherein the chromosome fragments comprise a second centromeric sequence functional in a plant cell.

42. (previously presented) A recombinant nucleic acid comprising a first centromeric sequence functional in a yeast cell, and a second centromeric sequence functional in a plant cell, isolated from the fused protoplasts of (d) in claim 37, or of (d) in claim 41, wherein said recombinant nucleic acid exhibits normal plant chromosomal activities.